NIET Journal of Engineering & Technology (NIETJET)

Volume 5, Issue Winter 2016 ISSN: 2229-5828 (Print)

Isolation of Lactic Acid producing Bacteria and production of Lactic Acid from Isolates

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Abstract*: Lactic acid is the most common and important chemical compound used in the pharmaceutical, cosmetic, chemical, and food industries. Various attempts have been made to produce lactic acid efficiently from inexpensive raw materials. Lactic acid can be produced by various methods such as fermentation of sugars and food waste. In this way, lactic acid is an environmentally friendly product that has gotten a lot of attention in recent years. The strains were isolated from five separate samples of curd, kimchi, garlic, rice water, and mango peel for the study. The study discusses the generation of lactic acid from various culinary wastes, including potato peel, orange peel, sugarcane peel, garlic peel, and mango peel. In the future, more emphasis should be placed on achieving maximum productivity and yield. Purification methods must be efficient enough to increase output while reducing product loss.*

Keywords: de Mann Rogosa Sharpe (MRS), Lactic acid, Lactic acid bacteria (LAB).

1. Introduction

Lactic acid is one of the most often used hydroxyl carboxylic acids in industry. It is extremely important since it was utilised not only as a raw ingredient in the fermentation of many goods, but also in the culinary, pharmaceutical, and textile industries. Aside from its uses in the food, pharmaceutical, and chemical industries, it's also a popular flavouring ingredient, acidulant, and bacteria inhibitor. Its ability to retain water makes it a helpful cosmetic ingredient. Lactic acid is regarded as one of the most important hydroxyl carboxylic acids and is in great demand due to its diverse applications. In 2017, around 370000 metric tonnes of lactic acid were manufactured, and the need for lactic acid is constantly increasing every year (1). Bacteria and fungus are the two types of microorganisms that can produce lactic acid Lactic acid bacteria are Gram-positive, non-spore-forming cocci or rods that create lactic acid as the primary result of carbohydrate fermentation. Lactic acid bacteria play a vital part in a variety of fermented foods (2)(2). They are utilised to preserve edible food materials through fermentation of other raw materials such as rice wine, beer, rice cakes, and fish by creating organic acids to inhibit putrefactive microbes and pathogens, in addition to being the key component in kimchi and other fermented foods (3, 4).The idea behind using food waste biomass to produce lactic acid is to create a cost-effective and environmentally friendly process. Every year, a large amount of food is thrown out around the world. According to German research, 18 million tonnes of food are wasted every year, of which 10 million tonnes are avoidable. If these 10 million tonnes of food are dumped in 2.6 million hectors of fertile land, 2.18 million tonnes of CO_2 will be produced (5).

This research highlights the importance of developing an effective technique for converting discarded food into a useful product. Biotechnology is often used in waste food utilisation techniques. Most processed foods are first hydrolysed by hydrolytic enzymes such as amylase and protease, or microorganisms such as *Aspergillus awamori* and *Aspergillus oryzae* are utilised to release hydrolytic enzymes for food waste bioconversion. Fructose, glucose, different amino acids, free amino nutrients, and phosphate are abundant in this secretory hydrolysate (6). When given the right conditions, these nutrients in food waste promote the growth of lactic acid bacteria (LAB) and the formation of lactic acid. Lactic acid contains a wide range of antibacterial characteristics.

Lactic acid bacteria (LAB) are the most commonly used probiotic bacteria. Enhancement of the epithelial barrier, increased adhesion to intestinal mucosa, and concurrent inhibition of pathogen adhesion are all major probiotic mechanisms of action, as are competitive exclusion of pathogenic microorganisms, production of anti-microorganism substances, and immune system modulation (7, 8). Lactic acid generation is thus unimportant in the industrial world, but it is essential for our health. Thus, we have used various culinary wastes, including potato peel, orange peel, sugarcane peel, garlic peel, and mango peel for the production of lactic acid.

2. Materials and Methods

2.1 Production of lactic acid from food waste

The peels and scraps from raw veggies (potato, orange peel, mango peel, garlic, sugarcane) collected and grinded the foregoing wastes into a semi-solid paste by cutting them into small pieces. 100g of the ground paste was transferred in separate conical flasks with 100ml distilled water and incubate for 10 minutes at 121ºC. For the fermentation process, single colony strains inoculated in various flasks and incubate in an incubator orbital shaker at 25-37ºC.

2.2 Media and Sample Preparation

De Mann Rogosa and Sharpe (MRS) Agar Preparation

The medium was prepared by combining 5g proteose peptone, 5g peptone, 2.5g yeast extract, 10g dextrose, 0.5g tween-80, 1g ammonium citrate, 2.5g sodium citrate, 0.05g magnesium sulphate, 0.0025g manganese sulphate, 1g dipotassium hydrogen sulphate, and 6g of agar in 500 millilitres of distilled water. Thoroughly mixing was done and pH maintained at 6.5. Further, it was autoclaved for 15 minutes at 121°C.

Isolation of Lactic Acid Bacteria

Lactic Acid Bacteria (LAB), which can be found in curd, rice water, kimchi, garlic, and mango peel were isolated from a variety of fruits, vegetables, and dairy products. Samples were serially diluted up to 10^{-9} in distilled water for isolation, then spread plated on deMann Rogosa Sharpe (MRS) agar plate and incubated anaerobically at 37ºC for at least 2 days. The isolated colonies were transferred to MRS broth and streaked three times on MRS agar to purify them. Further, the individual bacterial colonies were saved for the study.

2.2 Screening of Lactic Acid Bacteria

Indole Test

Tryptophan broth was used to inoculate of strains and incubation was provided for 24 to 48 hours at 37°C. Then, 0.5 ml of Kovac's reagent added to the broth culture.

Methyl Red-Voges Proskauer Test

Methyl Red-Voges Proskauer (MR-VP) broth was prepared by mixing 0.1g methyl red and 300ml 95% ethyl alcohol in 200ml distilled water. For the MR-VP tests, we inoculated the separate test tubes containing MR-VP broth with pure microorganism culture and incubated for up to 4 days at 35°C. For the VP test tubes, 5 drops of the methyl red indicator solution and 10 drops of Barrit's reagent A and B were used for the MR test.

To the VP test tubes, we added 0.6ml naphthol, followed by 0.2ml of 40% potassium hydroxide (KOH). The tubes were gently shake and left it undisturbed for 10-15 minutes.

3. Results and Discussion

Kimchi, Rice Water, Mango Peel, Garlic, and Curd were used to extract the 11 typical isolates. The isolates were then thoroughly investigated for a variety of biochemical and physiochemical tests. The staining was same for all *Lactobacillus species*. Short rod to coccobacilli with single, pair, and chain arrangement with various colony shape in MRS or Rogosa agar and in MRS slant with Gram negative, non-capsulated, non-sporing, non-acid fast and microscopic characteristics (Figure-1). Some biochemical assays, and physiological features were used to distinguish isolated and preliminary identified *Lactobacillus* strains (Figure-2-4).

All of the isolates studied were cultured at 37-45ºC, according to biochemical characterizations. In response to several testing, all of the isolates yielded distinct results. On the basis of the Carbohydrate fermentation test, isolates were further identified *(data not shown)*.

The properties of Kimchi, Mango Peel, Curd, Rice Water and Garlic were determined to be similar to the *Lactobacillus* spp. standard characteristics. The isolates, *Lactobacillus's* sake, *Lactobacillus mesenteroides*, *Lactobacillus caesi*, *Lactobacillus lactis*, *Lactobacillus bulgaricus*, and Bifidobacterium Longum, were identified respectively.

4. Conclusion

Chemical, petrochemical, agricultural, and food industries all contribute to environmental pollution. For adequate environmental regulation, many waste treatment procedures are used. Probiotic lactic acid bacteria have been demonstrated in studies to control intestinal flora balance, restrict cholesterol absorption, act as an antioxidant, and also reported to prevent tumours and cancer (9).

The major goal of this study was to identify LAB from various sources and produce lactic acid utilising the isolates. Five distinct samples of curd, kimchi, garlic, mango peel, and rice water yielded a total of 11 LAB strains. The strains were put through biochemical tests. Six groups of LAB isolates *Lactobacillus* sake, *Lactobacillus mesenteroides*, *Lactobacillus caesi*, *Lactobacillus lactis*, *Lactobacillus bulgaricus*, and *Bifidobacterium Longum* were identified. To make lactic acid, bacteria were isolated from curd, garlic, kimchi, sugarcane, and mango peel. The mixing of all the samples within 21 days produced almost 70 yields. As a result of the research, successful and highly efficient manufacturing of lactic acid from food wastes at a significantly lower cost was discovered (10–17).

With the help of lactobacillus, we were able to determine that food waste can be used to produce industrially important lactic acid. This is applicable to the pharmaceutical, chemical, and food industries. Lactic acid bacteria have probiotic potential and are useful in the production of food and biopharmaceuticals (18–20).

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Figure’s Legends

Figure-1: Single Cell Colony of (a) Kimchi, (b) Rice water, (c) Mango Peel, (d) Garlic, and (e) Curd.

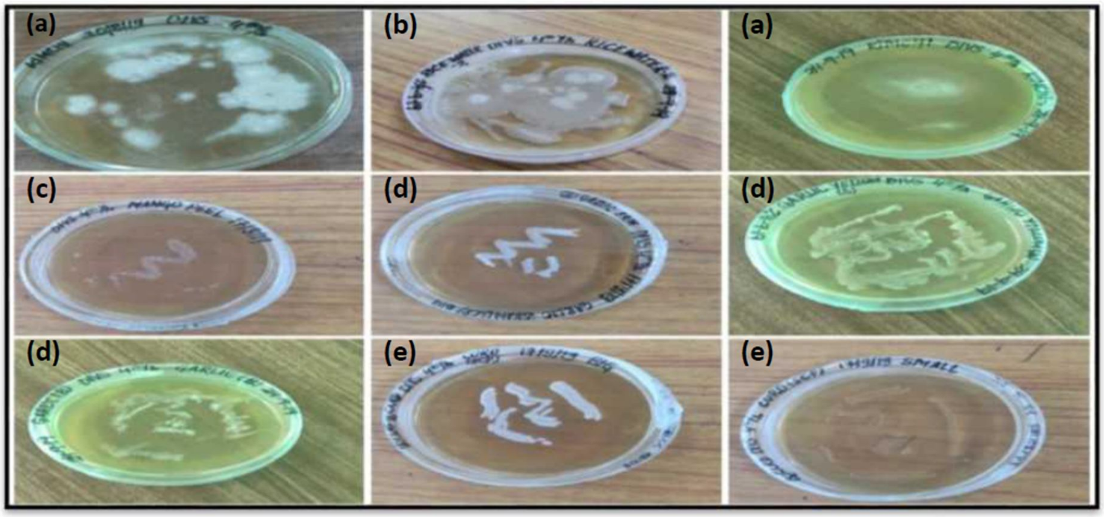
Figure-2: Indole test results of (a) Kimchi, (b) Rice water, (c) Mango Peel, (d) Garlic, and (e) Curd. the pink colour indicates a positive result, whereas no colour change indicates a negative result.

Figure-3: Methyl red test results of (a) Kimchi, (b) Rice water, (c) Mango Peel, (d) Garlic, and (e) Curd. The colour changes to red, indicating a positive outcome.

Figure-4: Voges Proskauer test results of (a) Curd, (b) Kimchi, (c) Garlic, and (d) Rice water. A positive result is indicated by the development of red colour, whereas a negative result by the development of a copper-like colour, potentially leading to false positives.

Figures

1.



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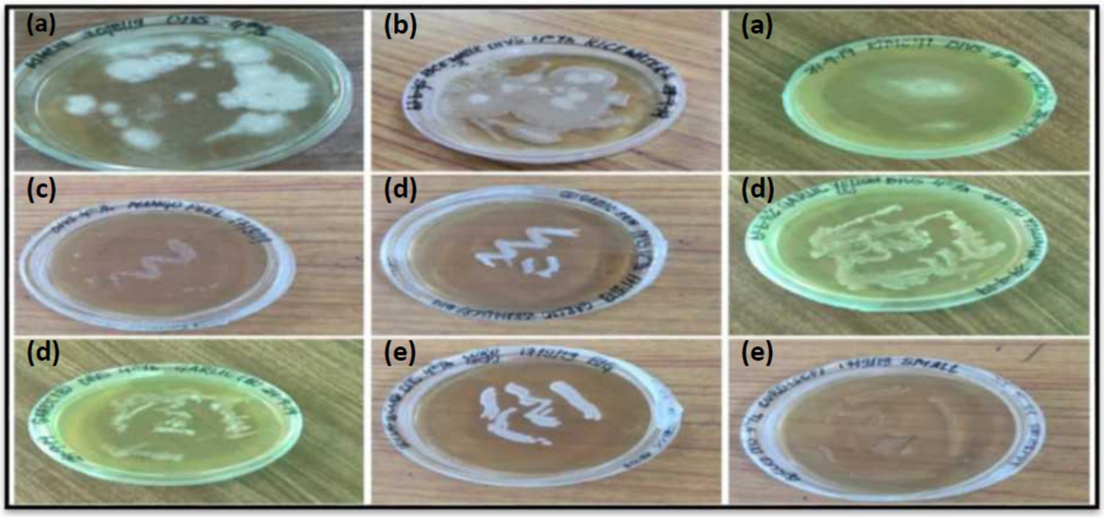

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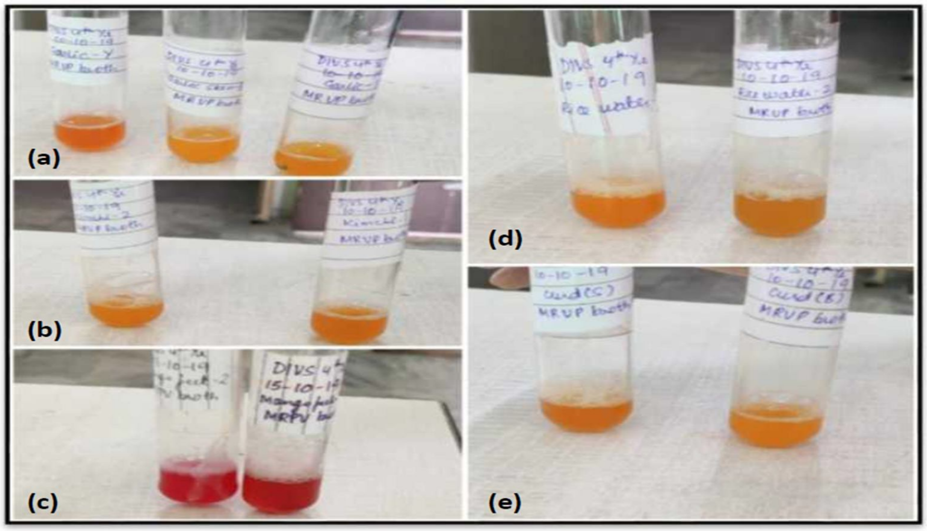

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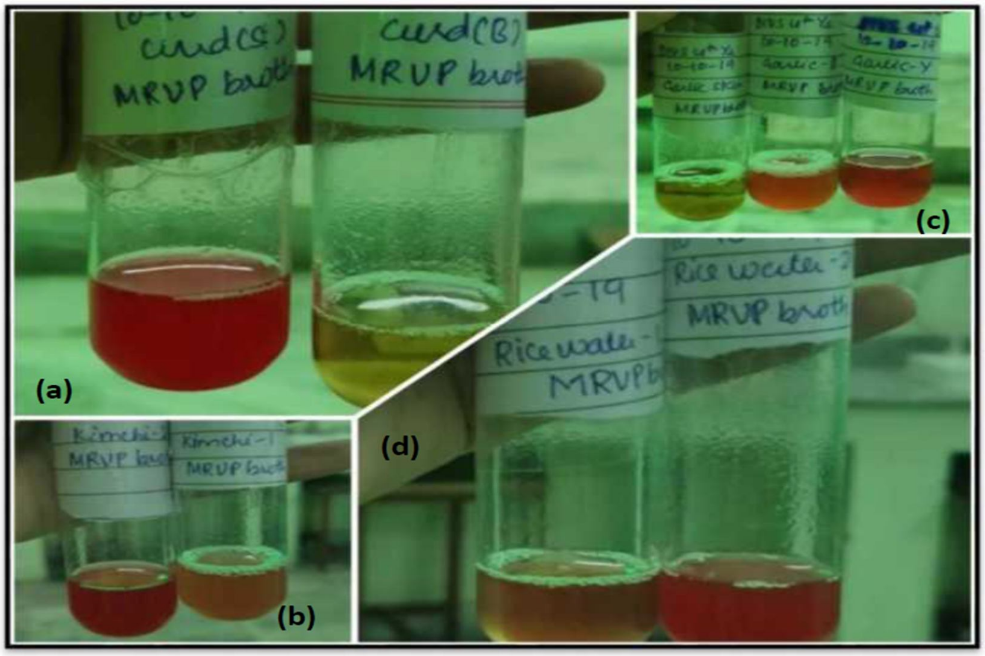
curd(S)
MRUP broth
curd(B)
MRUP broth
(a)
Kimchi-2
MRVP broth
Kimchi-1
MRUP broth
(b)
Garlic-Y
MRVP broth
(c)
Rice water-2
MRVP broth
Rice water-1
MRVP broth
(d)